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Article

Testing antimicrobial properties of common household spices in a real-world scenario

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SUMMARY
Proper disinfection of household items is essential to prevent illness, especially with respect to kitchen items. However, many currently used household cleaners are toxic. Accidental poisonings from common chemical cleaners thus represent a significant threat to humans and have been exacerbated by the pandemic. Thus, there is a pressing need to find safer alternatives to these cleaning products. Spices could be useful in this respect, given their relative safety when ingested, wide availability, and potent antimicrobial properties. To date, studies have tested antimicrobial properties of a variety of spices in conditions that are different from how they would be implemented as cleaners. We therefore sought to test antimicrobial properties of spices in a real-world scenario, by quantifying microbial growth before and after cleaning a cutting board with garlic, nutmeg, or paprika. These spices were found to be relatively ineffective at cleaning cutting boards, as evidenced by similar levels of microbial growth before and after cleaning, though some of these effects appeared to be microbe and spice dependent. Overall, we recommend caution with the currently described method of repurposing spices for cleaning purposes but encourage further work to this end.

INTRODUCTION
Kitchens contain some of the highest amounts of bacteria in a household, requiring proper cleaning procedures to limit illnesses (1). Unfortunately, many household cleaners are toxic and account more than 20% of calls to poison control centers (2,3). These poisonings are particularly harmful to young children and the elderly. The most common exposure route is orally, and sometimes involves exposure through contaminated kitchen utensils that are used to mix or store cleaning products (2,3). Poisoning by household cleaners has been exacerbated by the COVID-19 pandemic, as the Centers for Disease Control reported a 20% increase in calls to United States poison centers at the beginning of the pandemic (4). Prevention and awareness programs could be effective at reducing the incidence of these unintentional poisonings, but another alternative is to use non-toxic cleaning products.

Spices have been of large interest in this respect, given their safety even at moderate doses and when ingested, relatively low cost, and high availability (5,6). Studies have shown that many spices have antioxidant and anti-inflammatory properties in a variety of model organisms (5,7). Of note, two recent studies show that many spices have the ability to inhibit microbial growth, further supporting their repurposing for use as cleaners (8,9). However, most studies, including the previous two, test the antimicrobial properties of spices in conditions that are far different from real world scenarios. For example, Gehad and Springel showed antimicrobial properties of clove, cinnamon, garlic, sage, and thyme, but only against the gram-negative bacteria *Escherichia coli* (8). Meanwhile, our previous study asked whether spices could prevent microbial growth rather than addressing whether they could be utilized to clean surfaces (9). Other studies only test the antimicrobial properties of the active ingredient in a given spice (10). Thus, further study of spices is warranted, specifically in a context that is reminiscent of how they would be implemented in a real-world scenario.

We sought to test spices as household cleaners by culturing microbes from cutting boards before and after cleaning with a spice (garlic, nutmeg, or paprika) and water mixture. We chose to culture microbes from cutting boards based on previous evidence showing that they contain high amounts of microbes and because of their nearly universal place as a kitchen item (9). We chose to study garlic and nutmeg based on previous reports of their antimicrobial properties (8,9). Nutmeg and garlic contain different active ingredients, eugenol and allicin, respectively, which may confer different antimicrobial benefits (10-12). We also chose to test paprika, which has not been widely investigated for its antimicrobial effectiveness but contains large amounts of capsaicin, which has antimicrobial properties (13). We hypothesized, based on this evidence, that cleaning cutting boards with the spice and water mixtures would partially or completely prevent the growth of microbes. However, our data demonstrated minimal effects of spices at disinfecting common kitchen surfaces, though it appeared that the effects of garlic were dependent on the type of microbe. Our results advise caution when repurposing spices in the manner described here, while also encouraging future work to improve methods for such purposes.

RESULTS
We aimed to test the effectiveness of common, low-cost household spices as alternative cleaning products in a real-

world scenario. To this end, we swabbed cutting boards and culture microbes from them prior to cleaning, which served as our positive control. We then cleaned the cutting board with a mixture of spice (either paprika, garlic, or nutmeg) and water, and again swabbed and cultured microbes. Spices and their concentrations were based on their antimicrobial properties that have previously been described by us and others (8,9). After incubating for 48 h, we plated the microbes and allowed another 48 h of incubation. We demonstrated successful isolation of microbes from cutting boards, as illustrated by the visible growth on positive control plates **(Figure 1)**. We then took pictures of our plates and manually outlined microbial growth in ImageJ to obtain the percent area covered by cultured microbes. When analyzing each positive control plate, we observed quantitative and qualitative differences in the amount and appearance of microbes **(Figures 1 and 2)**. Due to these differences, we conducted further analysis in three pairwise comparisons, each with their own positive control. Importantly, samples came from cutting boards from three different households and thus likely contained different microbial populations. We therefore did not analyze differences in spice effectiveness between households. We did not observe any microbial growth on negative control plates, indicating that they were not contaminated during the experimental process **(Figure 1)**.

To quantify the ability of spices to clean a surface when mixed with water, we quantified the amount of microbial growth before and after cleaning a cutting board with a mix of 5 mL ultrapure water and 0.25 tsp of paprika, garlic, or nutmeg. We observed negligible effects of spices to hinder microbial growth following cleaning. In fact, only paprika was able to reduce the amount of microbial growth from cutting board cultures, but only by 3% **(Figure 2A)**. On the other hand, we unexpectedly noticed a slight increase in the amount of microbial growth following cleaning with garlic (approximately 2%), and a 10% increase in microbial growth following cleaning with nutmeg **(Figures 2B and C)**.

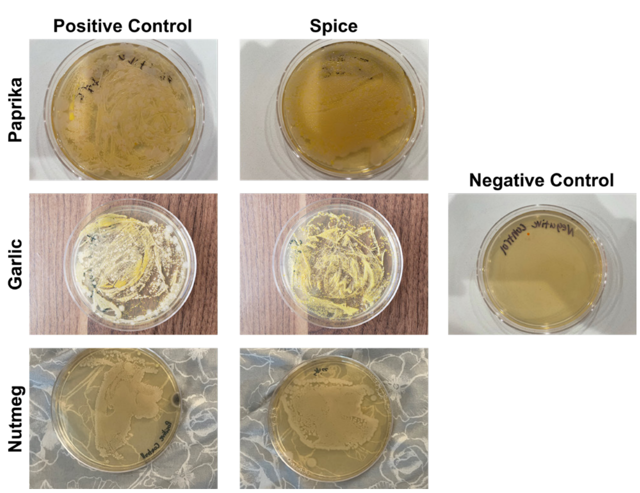

Figure 1. Representative images of plates growing microbes before and after cleaning cutting boards with spices. Microbes were cultured from samples obtained from a cutting board before (positive control) and after cleaning with a spice and water mixture. Cultured microbes were spread onto plates 48 h after beginning the cultures and images were taken 48 h after spreading. A representative image of a negative control plate is also included.

Interestingly, we also noticed qualitative differences between our positive control and garlic plates. Specifically, the positive control plate that was run in tandem with garlic demonstrated the growth of microbes that were yellow and white **(Figure 1)**. On the garlic plate, we only noticed the presence of yellow microbes **(Figure 1)**. We did not observe qualitative differences between positive controls and either the paprika or nutmeg plates, suggesting variability in microbes cultured between households **(Figure 1)**.

DISCUSSION

Here, we proposed a proof-of-concept study that sought to test the ability of spices to be repurposed as household cleaners. The spices and concentrations used in the present study were based on previous literature, which demonstrated their effectiveness as antimicrobial agents (8,9). We also chose to culture microbes from cutting boards based on the commonality within a household and propensity to harbor microbes (9). In stark contrast to other reports, we chose to test antimicrobial efficacy of these spices in a real-world cleaning scenario in an attempt to directly translate our results to the household. We hypothesized that each of these spices would be able to clean cutting boards, which was operationalized as lower or a lack of microbial growth compared to the positive control plate.

Surprisingly, garlic, nutmeg, and paprika, upon initial analysis of our data, were completely ineffective at preventing microbial growth. Only paprika was able to inhibit the growth of microbes cultured from cutting boards, but this effect was largely negligible (~3%). Meanwhile we observed a slight increase in the amount of microbes cultured following cleaning with garlic. Finally, we observed a 10% increase in the amount of microbes grown following cleaning with nutmeg.

Quantitative measures did not fully describe the antimicrobial effectiveness of the spices tested. We observed stark qualitative differences in the types of microbes cultured before and after cleaning, specifically in the case of garlic. On the positive control plate, we observed growth of both white and yellow microbes. Following cleaning with garlic, we noticed that the appearance of white microbes was drastically reduced, while the yellow microbes were unaffected. This suggests that antimicrobial properties of garlic may be specific to certain types of microbes, but further investigation is necessary to confirm this. While we observed no qualitative differences in microbial growth on other plates, this microbe-specific effect of garlic indicates that mixing spices may be necessary to obtain maximal benefits for cleaning purposes. Moreover, we utilized spices that were in pre-ground powder form, but some studies show benefits to different methods of preparation or even using fresh spices, specifically garlic, which warrants further study to determine the optimal form of spice to use for cleaning (12,14).

Our results were surprising, given that both garlic and nutmeg were previously found to completely prevent microbial growth from cutting board cultures specifically (8-11). However, we only tested a single cleaning regimen in the current study. For example, we chose to use 0.25 tsp of each spice based a previous study (9). However, many spices show an optimal concentration for antimicrobial properties, and these can differ between spices (8). For example, garlic was found to show optimal inhibition of *E. coli* at a 1% concentration, similar to cinnamon, thyme, and sage (8). In contrast,

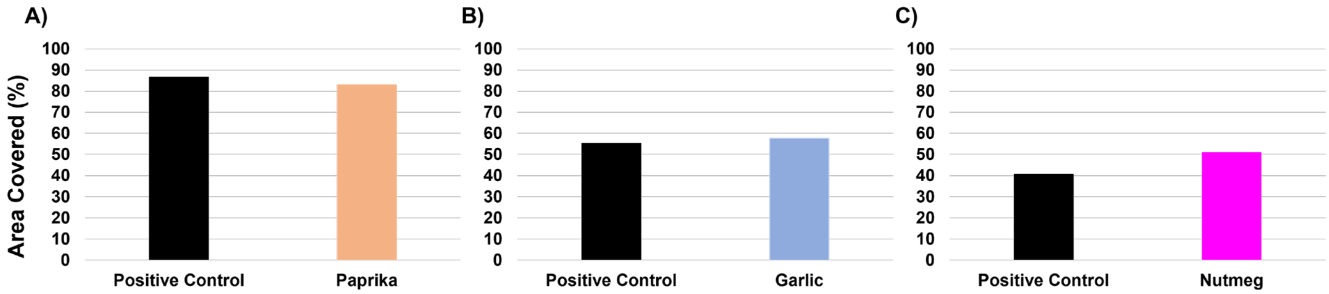

Figure 2. Quantification of microbial growth from cutting boards before and after cleaning with spices. Microbes were cultured before (positive control) and after cleaning cutting boards with spice and water mixtures using either **A)** paprika, **B)** garlic, or **C)** nutmeg. Microbial growth was quantified 48 h after plating on agar by manually outlining microbial growth in ImageJ.

oregano facilitated bacterial growth at a 1% concentration compared to lower concentrations (8). The active ingredients and mechanisms of antimicrobial properties are also worth considering in future studies considering combinations of spices. Eugenol, found in nutmeg, contains a free hydroxyl group that is thought to inhibit ion and ATP transport in addition to throwing off normal membrane permeability (11). Meanwhile, garlic contains high amounts of allicin, which appears to disrupt thiol-containing bacterial enzymes (12). Finally, it is unclear the exact antimicrobial mechanism of paprika and other capsaicin-containing compounds, but it could be due to interference with microbial biofilms (13). However, these diverse sets of active ingredients could be beneficial when considering which spices to combine into a single cleaning solution.

Our study has a few limitations that are worth stating. Due to limited resources, we were unable to run duplicate and/or triplicate plates. Replicates would have allowed for statistical analysis of our data, which would help determine whether small differences in growth were statistically and/or biologically meaningful. For similar reasons, we were unable to run plates that only contained spices. These experiments are important for determining whether spices were contaminated with microbes prior to use. Specifically, the qualitative differences we saw with garlic could be a result of microbes present in the garlic rather than being driven by those swabbed from the cutting board. Inclusion of a plate incubated following cleaning with household cleaners (bleach, ethanol, etc.) would have been useful to compare the efficacy of spices versus traditional cleaning methods. Differences in types of microbes cultured between locations and lack of ability to identify these microbes makes comparison with previous studies more difficult. Each culture was taken from a household with a different cutting board that varied in size, amount of use, and material (wood, plastic, etc.), which likely contributed to the variability in microbes cultured and could impact the effects of spices on microbes. Subculturing would have provided a better idea of whether a spice was effective against a single type of microbe, but as our main mission was to test these spices in a real-world scenario, we decided to not isolate single microbes by subculturing.

There are a number of future studies that are warranted given our present results. The systematic investigation of different types of spices should be considered, in addition to their concentrations and solvent medium. Altering exposure time of spice mixtures on the surface to be cleaned is another variable worth considering. Another interesting follow up study would be to determine the time course of microbial growth, but this would be more suitable once a proper spice cleaning mixture is identified. Furthermore, our plates appeared overgrown, even after 48 h post-incubation, which limited our ability to count microbial colonies and perhaps uncover more subtle differences in microbe growth (i.e. number of colonies and average colony size). We based our methods on a previous report, but further diluting these samples, shortening incubation time, and/or recording growth prior to 48 h would improve our ability to quantify microbial growth (9). Nonetheless, through our study, we demonstrated minimal effectiveness of spices as cleaning products in a real-world application. We therefore caution the use of spices as household cleaners under the currently studied application but encourage future work that refines these methods for such purposes.

MATERIALS AND METHODS

Preparation of spices

The spices that we used were garlic (Great Value), paprika (House of Spices), and nutmeg (McCormick), which were pre-ground. Cleaning solutions were prepared by adding 0.25 tsp of a spice to 5 mL of ultrapure water and mixing by inverting the tube. This concentration of spice was based on a previous report, with the distinction that spices here were added to a cleaning mixture, rather than directly to agar plate media (9).

Swabbing and culturing

Sterile swabs were submerged in ultrapure water and used to swab a 2 in by 2 in spot on a cutting board. The swab was then placed into a tube containing 5 mL of tryptic soy broth and served as our positive control, prior to cleaning with spice and water mixtures. We applied 1 mL of the spice solution on the cutting board, which was centered around the previously swabbed spot. The mixture was allowed to sit for approximately 10-15 s and was then cleaned dry with a paper towel. The same spot was then swabbed again with a new sterile swab submerged in ultrapure water and subsequently placed in a tube containing 5 mL of tryptic soy broth. Both tubes were left for 48 h to incubate at room temperature.

Agar plate preparation and microbial spreading

Solid agar was microwaved in 30 s intervals for approximately 2 min until liquified. The liquified agar was used to fill petri dishes and allowed to solidify. After 48 h of culturing, a single drop of the positive control and spice and water mixture liquid culture were placed into separate fresh vials of 5 mL of tryptic broth. A single drop (approximately 0.05 mL) of these diluted mixtures were placed in the center of agar plates using a transfer pipette and were spread evenly over the surface of the plate. Plates were covered and incubated for 48 h at room temperature. Nothing was spread onto the negative control plates to ensure proper sterile technique throughout the experimental process.

Quantification of microbial growth

Images of each plate were taken 48 h after plating. Images were subsequently imported into ImageJ for further analysis (15). Colonies of microbes were manually outlined using the tracing tool and the measure function was used to calculate the amount of area covered by microbes. This was then converted to a percentage of the total plate area and graphed in Excel.

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